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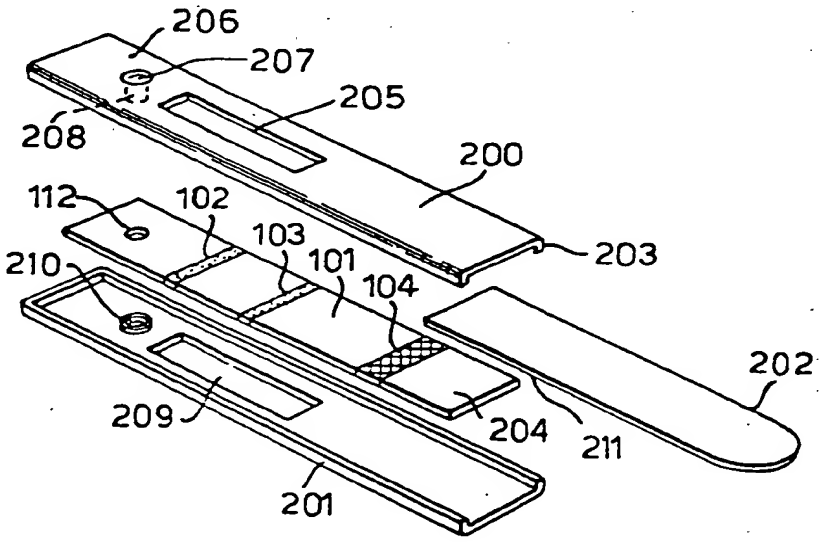
(54) Title: ASSAY DEVICES AND THE MANUFACTURE THEREOF

(57) Abstract

An assay sample testing device comprises a porous liquid-permeable carrier strip within a casing, the carrier including a detection zone in which an assay result is revealed by specific binding of a detectable material directly or indirectly to a binding agent immobilised in the detection zone, the casing including internal registration means which cooperatively engages with corresponding registration means associated with the carrier, for example a pin and hole, such that said detection zone within the casing is precisely located in relation to the interlocking means on the casing. During manufacture, the corresponding registration means is used to control accurate formation of the detection zone on the carrier, and accurate placement of the carrier within the casing.



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(74) Agent: BUTLER, David, John; Unilever PLC, Patent Division, Colworth House, Sharnbrook, Bedford MK44 1LQ (GB).

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Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ASSAY DEVICES WITH NON-WOVEN SAMPLE COLLECTION ZONE

(57) Abstract

An assay device comprises a sample-collecting wick made from non-woven fabric material laminated to plastics sheet. Preferably, the fabric is a 30:70 blend of viscose and polyester, and has a fibrous structure in which more than two-thirds of the fibres run substantially parallel to the intended direction of liquid flow in the wick.

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(71) Applicant: UNIPATH LIMITED [GB/GB]; Wade Road, Basingstoke, Hampshire RG24 0PW (GB).

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(54) Title: ASSAY DEVICES

(57) Abstract

Storage stability of an assay device, comprising an assay strip and sensitive reagents such as antibodies within a plastics casing, is maintained by moulding some or all of the casing from desiccant-containing plastics material, especially a blend of about 60-65 % polystyrene and about 30 % silica dust. Ideally the desiccant-containing plastics material is used in the moulding of a removable cap for the device. The cap can be made by sandwich injection moulding, using the desiccant-containing polystyrene as a core, surrounded by conventional polystyrene.

hermetic. However, the sample receiving member should be sufficiently enclosed by th cover means to prevent gross loss of moisture by dripping or liquid flow into ext rnal objects such as fabrics into which the non-enclosed sample receiving member might come into contact. In addition, the likliehood of loss of moisture by evaporation into the surrounding atmosphere from the sample receiving member should be substantially reduced under normal conditions of temperature and humidity likely to be encountered during use. Preferably, to the extent that this is practicable using conventional materials such as plastics and conventional moulding techniques, the sealing of the sample receiving member by the cover means should be essentially hermetic.

At the present time, commercially available home-use diagnostic devices generally provide the assay result in qualitative or semi-quantitative terms only. For example, a pregnancy test only needs to give a "yes/no" result. However, there is a desire to adapt the home-use technology to more sophisticated uses. An example is in the monitoring of the human ovulation cycle to provide more accurate status information which can be used to assist conception, or indeed to facilitate contraception. This can best be achieved on the basis of accurate numerical data. Proposals have been made to utilise changes in analyte levels in body fluids; especially hormone metabolites in fluids such as urine and saliva, to indicate the changing status of the ovulation cycle. Frequent, eg. daily, testing of body fluid samples is needed. Calculation and interpretation of the individual assay results typically require the use of an electronic device such as a microprocessor. It is generally envisaged that whereas the microprocessor or similar unit will be retained for a long time by the user, the testing of body fluid samples will be conducted using a plurality of disposable testing devices which are used only once and then thrown away.

Hence, during the repetitious assay procedure, a disposable analytical device will be used to sample the body fluid and to perform the basic assay. Thereafter the user will present the disposable device to the permanent reader/microprocessor in order for the individual assay result to be recorded and interpreted. Typically this may involve engagement or insertion of a portion of the disposable device into a slot or other shaped orifice in the structure of the reader/microprocessor, to facilitate positive location of the disposable device in relation to a reading head which forms part of the reader/microprocessor. Because the reader/microprocessor is an item of equipment which the us r must retain for repeated use, it is easy to imagine that the user may have misgivings about such a proc dure. The exterior of the disposable analytical device may easily become contaminated with sample fluid. Such contaminating fluid might be transferred to the permanent reader/microprocessor, and for reasons of hygien there may be reluctance to retain and re-use such a contaminated item. We believe that in order to promote consumer acceptance of such an analytical system, it is necessary to minimize the likelihood of sample contamination on the portion of the disposable assay device which is to be engaged with the reader/microprocessor.

Moreover, if body fluid is transferred therefrom into the reader/microprocessor, it may interfere with the accurate performance of the reader/microprocessor, especially if it accumulates therein or dries thereon to leave a deposit that is difficult to remove.

If an analytical device of the invention is to be used in conjunction with a separate reading device, such as an electronic reader than can interpret the result of an assay performed within the analytical device, it is convenient if said window means is located in a portion of said casing which is cooperatively engageable with means for reading the result of the analysis, said cooperatively engageable portion becoming exposed when said cover means is released to expose said window means.

The invention also provides advantageous ways of obtaining and assaying sample liquids, such as body fluids, using an analytical device according to the invention as described above, wherein during sample collection involving introduction of said bibulous sample receiving member into a source of sample liquid said device is hand-held by means of said cover means enclosing said window means, and thereafter said cover means is released to expose said window means. Preferably, after release of said cover means to expose said window means, the same cover means is used to enclose said bibulous sample receiving member. Preferably, following sample collection and enclosure of said bibulous sample receiving member, said device is hand-held by means of said cover means enclosing said bibulous sample receiving member, for example to facilitate presentation of said window means to means for reading the result of said analysis.

By way of example only, a preferred embodiment of the invention is now described with reference to the accompanying drawings, which show:

Figure 1: A general view of an analytical device in accordance with the invention together with an associated cap.

Figure 2: A view of the device of Figure 1 seen from above with the cap placed over the window means.

Figure 3: A vi w of the device of Figure 1, again seen from above, but with the cap removed and

INDIVIDUAL A

CYCLE A 1: Profile-establishing cycle

Day	Phase	E3G value	Actual Ovulation
1	infertile	2.7	
2	"	3.3	
3	"	2.4	
4	"	1.8	
5	"	3.6	
6	"	2.5	
7	"	1.7	
8	"	1.9	
9	"	3.1	
10	"	5.4	
11	"	2.1	
12	"	5.3	
13	"	10.5	
14	"	7.7	
15	fertile	5.2	
16	"	8.3	
17	"	6.8	
18	"	4.3	LHM + 1
19	"	4.9	
20	"	5.3	
21	postfertile		
22	"		
23	"		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Chosen testing commencement day for next cycle: 12

CYCLE A 2

Day	Phase	E3G value	Actual Ovulation
1	infertile	2.0	
2	"	1.5	
3	"	1.1	
4	"	4.1	
5	"	4.0	
6	"	3.5	
7	"	2.3	
8	"	1.9	
9	"	3.6	
10	"	3.7	
11	"	3.0	
12***	"	9.2	
13	"	8.9	
14	fertile	14.6	
15	"	12.6	
16	"	8.8	
17	"	15.8	LHM + 1
18	"	6.9	
19	"	6.5	
20	postfertile	6.5	
21	"		
22	"		
23	"		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Days in advance of actual ovulation: 5

Chosen testing commencement day for next cycle: 11

CYCLE A 3

Day	Phase	E3G value	Actual Ovulation
1	infertile	2.0	
2	"	2.0	
3	"	2.7	
4	"	2.9	
5	"	2.7	
6	"	1.6	
7	"	2.5	
8	"	5.4	
9	"	4.0	
10	"	5.6	
11***	"	3.7	
12	fertile	6.2	
13	"	23.6	
14	"	21.3	
15	"	8.3	LHM + 1
16	"	4.5	
17	"	3.7	
18	postfertile	3.4	
19	"	2.8	
20	"	3.1	
21	"		
22	"		
23	"		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Days in advance of actual ovulation: 4

Chosen testing commencement day for next cycle: 9

CYCLE A 4

Day	Phase	E3G value	Actual Ovulation
1	infertile	2.4	
2	"	2.8	
3	"	5.2	
4	"	3.6	
5	"	2.5	
6	"	3.1	
7	"	3.9	
8	"	4.6	
9***	"	5.1	
10	"	6.1	
11	fertile	16.7	
12	"	10.8	
13	"	22.8	
14	"	21.3	LHM + 1
15	"	9.4	
16	"	12.2	
17	postfertile	5.7	
18	"	5.2	
19	"	7.5	
20	"	9.0	
21	"		
22	"		
23	"		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Days in advance of actual ovulation: 5

Chosen testing commencement day for next cycle: 9

CYCLE A 5

Day	Phase	E3G value	Actual Ovulation
1	infertile	2.3	
2	"	2.8	
3	"	2.7	
4	"	2.3	
5	"	2.8	
6	"	4.8	
7	"	5.6	
8	"	4.5	
9***	"	3.2	
10	"	8.5	
11	"	7.3	
12	"	6.3	
13	"	7.0	
14	fertile	11.8	
15	"	19.3	
16	"	18.5	
17	"	9.2	LHM + 1
18	"	5.3	
19	"	4.8	
20	postfertile	6.1	
21	"		
22	"		
23	"		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Days in advance of actual ovulation: 8

Chosen testing commencement day for next cycle: 9

INDIVIDUAL B

CYCLE B1: Profile-establishing cycle

Day	Phase	E3G value	Actual Ovulation
1	infertile	10.9	
2	"	15.2	
3	"	21.2	
4	"	12.7	
5	"	11.8	
6	"	16.5	
7	"	15.6	
8	"	25.1	
9	"	10.1	
10	"	16.8	
11	"	28.2	
12	"	24.6	
13	"	28.7	
14	"	27.7	
15	"	62.6	
16	"	68.5	
17	fertile	61.9	
18	"	103.4	
19	"	85.4	
20	"	45.4	LHM + 1
21	"		
22	"		
23	postfertile		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Chosen testing commencement day for next cycle: 13

CYCLE B 2

Day	Phase	E3G value	Actual Ovulation
1	infertile	27.5	
2	"	28.8	
3	"	24.7	
4	"	22.6	
5	"	24.9	
6	"	28.9	
7	"	14.6	
8	"	8.4	
9	"	24.7	
10	"	33.6	
11	"	39.3	
12	"	25.6	
13***	"	43.2	
14	"	67.1	
15	fertile	62.0	
16	"	94.6	
17	"	58.4	LHM + 1
18	"	42.4	
19	"	60.4	
20	"	56.0	
21	postfertile		
22	"		
23	"		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Days in advance of actual ovulation: 4

Chosen testing commencement day for next cycle: 11

CYCLE B 3

Day	Phase	E3G value	Actual Ovulation
1	infertile	46.4	
2	"	30.0	
3	"	12.4	
4	"	6.5	
5	"	8.7	
6	"	17.2	
7	"	14.9	
8	"	11.8	
9	"	11.0	
10	"	13.1	
11***	"	25.6	
12	"	32.5	
13	"	23.9	
14	fertile	63.8	
15	"	22.1	
16	"	65.9	
17	"	41.2	LHM + 1
18	"	7.6	
19	"	35.3	
20	postfertile	33.7	
21	"		
22	"		
23	"		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Days in advance of actual ovulation: 6

Chosen testing commencement day for next cycle: 11 (no change)

CYCLE B 4

Day	Phase	E3G value	Actual Ovulation
1	infertile	17.7	
2	"	12.2	
3	"	7.2	
4	"	6.2	
5	"	13.9	
6	"	12.9	
7	"	12.7	
8	"	9.3	
9	"	16.5	
10	"	17.7	
11***	"	26.0	
12	"	38.3	
13	fertile	70.6	
14	"	74.6	
15	"	70.6	
16	"	49.7	LHM + 1
17	"	23.5	
18	"	29.8	
19	postfertile	44.4	
20	"	32.7	
21	"		
22	"		
23	"		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Days in advance of actual ovulation: 5

Chosen testing commencement day for next cycle: 10

CYCLE B 5

Day	Phase	E3G value	Actual Ovulation
1	infertile	33.9	
2	"	27.3	
3	"	20.2	
4	"	7.0	
5	"	12.7	
6	"	7.2	
7	"	14.5	
8	"	14.8	
9	"	10.8	
10***	"	8.7	
11	"	14.1	
12	"	17.4	
13	"	41.3	
14	"	57.5	
15	fertile	42.0	
16	"	55.4	
17	"	60.1	
18	"	39.4	LHM + 1
19	"	24.7	
20	"	10.5	
21	postfertile		
22	"		
23	"		
24	"		
25	"		
26	"		
27	"		
28	"		
29	"		
30	"		

Days in advance of actual ovulation: 8

Chosen testing commencement day for next cycle: 10 (no change)

CLAIMS:

1. A method of monitoring the status of a current ovulation cycle of an individual mammalian female subject, involving repeated testing of the body fluid concentration of at least one analyte of significance in relation to the status of the ovulation cycle during at least the pre-ovulation phase of the current ovulation cycle of the individual subject, wherein testing for said analyte concentration during the current ovulation cycle is commenced a plurality of days following the onset of menses but at least 2 numerical days in advance of the earliest numerical day on which actual ovulation has occurred in one or more previous ovulation cycles in the same individual subject.

2. A method according to claim 1, wherein testing is commenced at least 3 numerical days in advance of the earliest numerical day on which actual ovulation has occurred in one or more previous ovulation cycles in the same individual subject.

3. A method according to claim 1, wherein testing is commenced at least 4 numerical days in advance of the earliest numerical day on which actual ovulation has occurred in one or more previous ovulation cycles in the same individual subject.

4. A method according to any one of the preceding claims, wherein testing is commenced at least 5 numerical days following the onset of menses.

5. A method according to claim 1, wherein the testing is commenced on a numerical day in advance of the earliest numerical day on which actual ovulation has occurred in one or more previous ovulation cycles in the

same individual subject, said testing commencement day being calculated according to the following relationship:

Earliest previous ovulation day	Testing commencement day
8 - 10	-4
11 - 14	-5
15 - 18	-6
19 - 23	-7
24 - 28	-8
29+	-9

6. A method according to any one of the preceding claims, wherein the body fluid is urine.

7. A method according to any one of the preceding claims, wherein the analyte is estradiol or a metabolite thereof, such as E3G.

8. A method according to any one of the preceding claims, wherein 2 or more analytes are tested to provide comparative data, such as the concentration ratio of two analytes tested simultaneously.

9. A method according to any one of the preceding claims, wherein the earliest actual ovulation day is derived from data collected during at least 3 consecutive previous cycles.

10. A method according to any one of the preceding claims, wherein the earliest actual ovulation day is derived from data collected during at least 5 consecutive previous cycles.

11. A method according to any one of the previous claims, wherein the earliest ovulation day is derived from data obtained during at least the immediately preceding cycle.

12. A method according to claim 11, wherein the earliest ovulation day is derived from data obtained from a rolling reference base consisting of a fixed number of consecutive cycles immediately preceding the current cycle.

13. A method according to claim 12, wherein the rolling reference base consists of the immediately preceding 4 to 10 cycles.

14. A method according to claim 12, wherein the rolling reference base consists of the immediately preceding 5 or 6 cycles.

15. A method according to any one of the preceding claims, wherein actual ovulation day is determined by detecting the peak concentration of urinary LH.

16 A method according to any one of claims 1 to 14, wherein actual ovulation is determined by detecting the urinary LH surge.

17. A method according to any one of the preceding claims, wherein the subject is human.

18. A test kit for conducting a method according to any one of the preceding claims, comprising one or more testing devices for determining the concentration (in relative or absolute terms) of said at least one analyte in said body fluid, together with means enabling a user to derive a precise testing commencement day from knowledge of the numerical day on which actual ovulation occurred during at least one previous ovulation cycle.

19. An electronic means programmed for use in a method according to any one of claims 1 to 17.

20. A plurality of disposable body fluid testing devices, packaged together with instructions for use in a method according to any one of claims 1 to 17.

21. A plurality of disposable urine testing devices, from each of which the urinary concentrations of E3G and LH can be determined, packaged together with instructions for use in a method according to any one of claims 1 to 17.

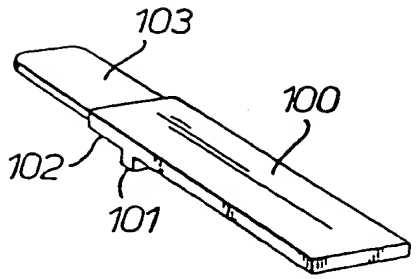
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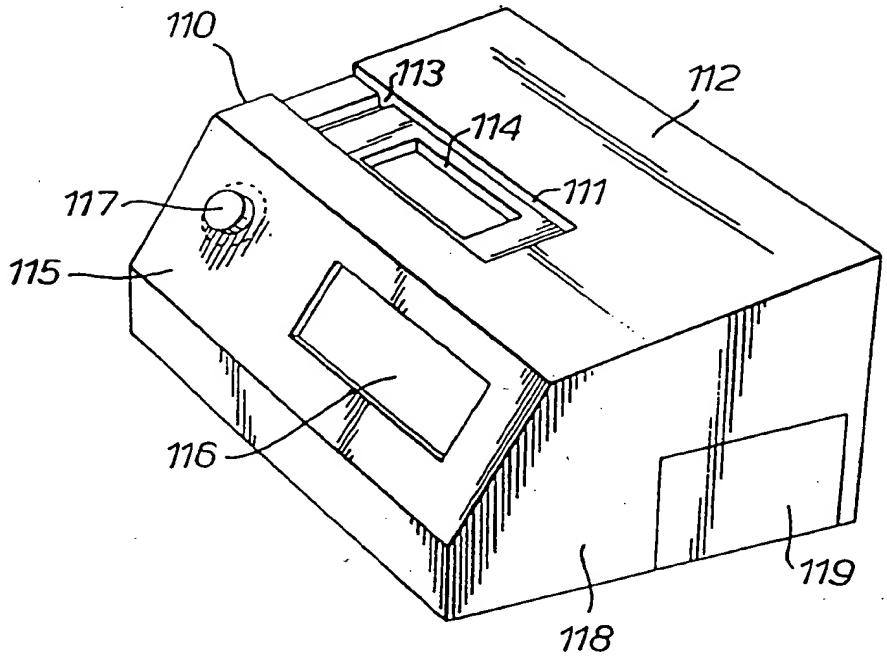
Fig. 1.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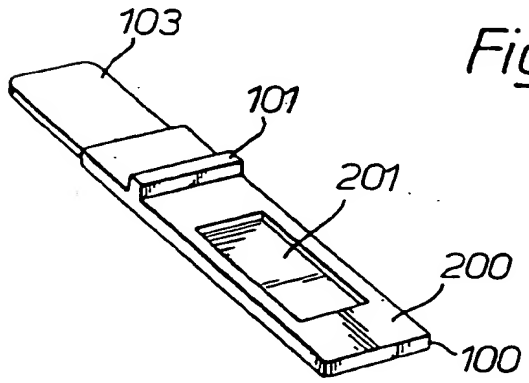
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Fig. 2.

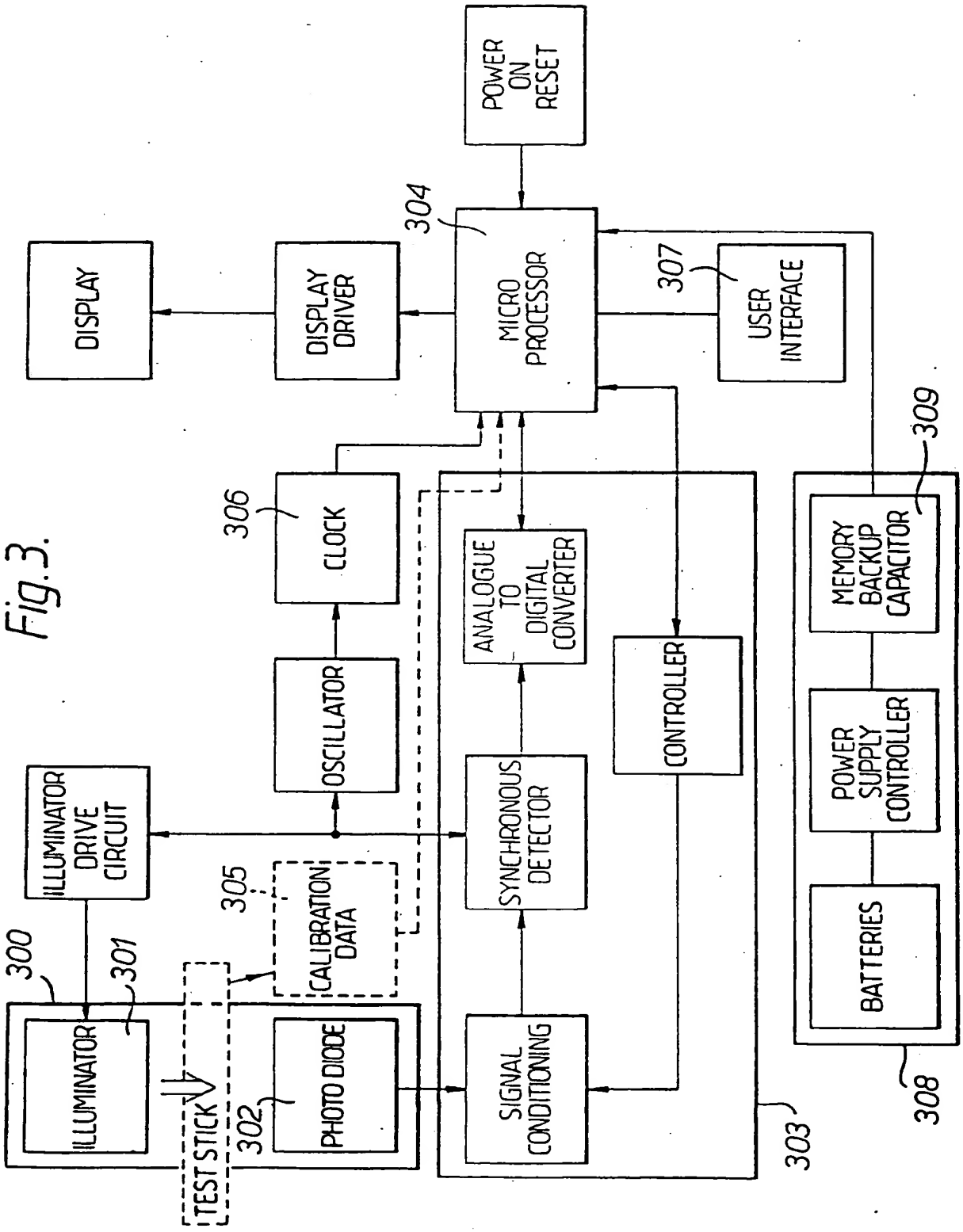
POWER ON RESET
304
DISPLAY
DISPLAY DRIVER
MICRO PROCESSOR
307
USER INTERFACE
309
306
CLOCK
ANALOGUE TO DIGITAL CONVERTER
MEMORY BACKUP CAPACITOR
OSCILLATOR
CONTROLLER
ILLUMINATOR DRIVE CIRCUIT
SYNCHRONOUS DETECTOR
POWER SUPPLY CONTROLLER
305
CALIBRATION DATA
300
301
BATTERIES
ILLUMINATOR
TEST STICK
302
PHOTO DIODE
SIGNAL CONDITIONING
303
308

Fig. 3.

INTERNATIONAL SEARCH REPORT

Intern[illegible]plication No
PCT/EP 93/02146

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 G01N33/74 G01N33/76 A61B10/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 G01N A61B G06C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CONTRACEPTION vol. 33, no. 4 , April 1986 , LOS ALTOS, CA, US pages 327 - 345 S.Z. CEKAN ET AL. 'The Prediction and/or Detection of Ovulation by Means of Urinary Steroid Assays' see the whole document ---	1-21
Y	HUMAN REPRODUCTION vol. 6, no. 4 , April 1991 , OXFORD, GB pages 515 - 518 P. BISCHOF, P.G. BIANCHI AND A. CAMPANA 'Comparison of a rapid, quantitative and automated assay for urinary luteinizing hormone (LH), with an LH detection test, for the prediction of ovulation' see the whole document --- -/--	1-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 December 1993	30. 12. 93
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Doepfer, K-P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 291 194 (UNILEVER NV) 17 November 1988 see the whole document & GB,A,2 204 398 (UNILEVER NV) cited in the application	18-21
Y	INTERNATIONAL JOURNAL OF GYNECOLOGY AND OBSTETRICS vol. SUPPL., no. 1 , 1989 , SHANNON, IE pages 111 - 122 J.B. BROWN, L.F. BLACKWELL, J. HOLMES AND K. SMYTH 'New assays for identifying the fertile period' cited in the application see the whole document	1-17
Y	INTERNATIONAL JOURNAL OF GYNECOLOGY AND OBSTETRICS vol. SUPPL., no. 1 , 1989 , SHANNON, IE pages 35 - 43 W.P. COLLINS 'Biochemical indices of potential fertility' see the whole document	1-17
A	FERTILITY AND STERILITY vol. 47, no. 2 , February 1987 , BIRMINGHAM, ALA., US pages 259 - 264 MICHAEL VERMESH ET AL. 'Monitoring techniques to predict and detect ovulation' see the whole document	1-21
A	FERTILITY AND STERILITY vol. 44, no. 3 , September 1985 , BIRMINGHAM, ALA., US pages 328 - 334 LARS E. M. SCHIPHORST, WILLIAM P. COLLINS, J. PATRICK ROYSTON 'An estrogen test to determine the times of potential fertility in women' see the whole document	1-21
A	STEROIDS vol. 31, no. 2 , February 1978 , SAN FRANCISCO US pages 175 - 187 S.M. JUDGE ET AL. 'Time-course relationships between serum LH, serum progesterone and urinary pregnanediol concentration in normal women' see the whole document	1-21

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	INTERNATIONAL JOUNAL OF FERTILITY vol. 30, no. 3 , March 1985 , LAWREENCE, KANSAS, US pages 18 - 30 WORLD HEALTH ORGANISATION, GENEVA, CH 'A Prospective Multicentre Study to Develop Universal Immunochemical Tests for Predicting the Fertile Period in Women'	
A	SCIENCE vol. 248 , 1 June 1990 , LANCASTER, PA US pages 1061 - 1062 CARL DJERASSI 'Fertility Awareness: Jet-Age Rhythm Method?'	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No
PCT/EP 93/02146

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0291194	17-11-88	AU-B-	626207	23-07-92
		AU-A-	1622888	02-12-88
		DE-U-	8805565	18-08-88
		EP-A-	0560410	15-09-93
		EP-A-	0560411	15-09-93
		FR-A-	2614423	28-10-88
		WO-A-	8808534	03-11-88
		GB-A-	2204398	09-11-88
		JP-T-	1503174	26-10-89
		AU-A-	1704992	27-08-92
		AU-A-	1705092	27-08-92
GB-A-2204398	09-11-88	AU-B-	626207	23-07-92
		AU-A-	1622888	02-12-88
		DE-U-	8805565	18-08-88
		EP-A-	0291194	17-11-88
		EP-A-	0560410	15-09-93
		EP-A-	0560411	15-09-93
		FR-A-	2614423	28-10-88
		WO-A-	8808534	03-11-88
		JP-T-	1503174	26-10-89
		AU-A-	1704992	27-08-92
		AU-A-	1705092	27-08-92

specificity of single type possessed by monoclonal antibodies, and describes their production. As to applications, the review mentions their uses f r recognising surface-antigens on, for example, egg cells and viruses, and in therapy of cancer and management of patients carrying organ transplants.

Cited specification GB—2 013 211 discloses the use of $F(ab')_2$ fragments of immunoglobulins adsorbed to latex particles in immunoassays in place of native immunoglobulins themselves.

According to this invention we provide a process for carrying out an immunoassay which comprises bringing together for reaction in a single incubation mixture:

(a) a sample under assay, possibly containing a substance being tested for,

(b) an unlabelled specific binding partner for the substance, immobilised on a solid support, and

(c) a labelled specific binding partner for the substance,

characterised in that competitive interference between the binding reactions of the substance being tested for and reagents (b) and (c) is avoided by the use in components (b) and (c) of two monoclonal antibodies of narrow and different, non-interfering specificity with respect to the same antigen under test.

In a useful embodiment of the process, component (b) can comprise a stick, peg or stud for dipping into a liquid assay reagent, and can have an immunosorbent surface.

The narrow specificity required of the monoclonal antibody is a capacity to bind specifically with the substance under test but without preventing the binding reaction between the substance under test and its other specific binding partner. Such a monoclonal antibody can be selected out of a number of monoclonal antibodies with an affinity for the substance under test, by using normal methods to verify the progress of a binding reaction between the other specific binding partner and a complex previously formed between the substance to be tested in the assay and the monoclonal antibody to be selected.

Monoclonal antibody of sufficiently narrow specificity can, for example, be produced in known manner as antibody derived from a line of antibody-producing cells, derived from a single antibody-producing progenitor cell or cells. Such a line can, for example, be produced by known cell fusion, culture and isolation techniques using very pure antigens as comparative material.

In a "sandwich" test configuration, antigen under test can be specifically adsorbed to a first antibody bound to a solid surface, and a second antibody carrying an enzymic or other (e.g. fluorescent or radioactive) marker is specifically bound to be absorbed antigen under test. Marker specifically so bound is used for measurement and determination f the antigen under test, e.g., by direct measur ment, such as radiometry or flu rimetry, or exp sur of enzym marker t substrate followed by product measurement. Thus, in preferred sandwich tests, the two antibodies used can have different, non-interfering specificity with respect to the same antigen under test.

If antibodies from ordinary antisera raised against unmodified antigen (polyclonal antibodies) are used in sandwich tests, there is a very likely risk that if all ingredients are mixed in a single step there will be interference between the two specific adsorption reactions. When such tests are carried out according to the present invention, using apparatus as described herein, such interference can be avoided by using antibodies of narrow specificity as described. Our divisional European Patent Application No. 83111532.4 concerns alternative means for avoiding such interference, by ensuring that the binding of test material to the solid surface takes place before exposure of test material to the other (marker-conjugated) binding agent if there is a risk that binding by that other agent would prevent subsequent adsorption to the solid surface: according to the divisional application, such a sequence can be ensured by arranging for slow release of the other (marker-conjugated) binding agent.

Particular instances of suitable assay specificities and antibody specificities are described for example below.

It has also been found that in carrying out such specific binding assays, a worthwhile improvement in reaction kinetics can be obtained if the reaction liquid containing ingredients (a), (b) and (c) is contained in a well or cup of which the majority of the volume is occupied by a displacer body. (The use of various rod or ball shaped forms is known in connection with other kinds of immunoassay, as described in G.B. Specification Nos. 1,414,479 and 1,485,729).

The displacer body can, for example, be of a shape substantially complementary to and slightly smaller than that of the cup or well, so that the liquid phase containing one of the specific binding reagents is approximately in the form of a shell occupying the space between the displacer and the cup or well. The displacer can be loose-fitting and not fixedly mounted, i.e. movable relatively to the cup or well, so that by relative motion between displacer and well the liquid between them can be given a stirring or agitation motion.

For example, a round well can have a round displacer therein with an external diameter slightly smaller than the diameter of the well. The presence of the displacer can reduce the space available for liquid in the well by a factor of for example 2—10, e.g. 3—8, comparing volumes based on similar liquid levels in the well, e.g. when filled to its normal operating level, or its maximum capacity. For example, a microtitre well designed to have 300 microlitre of liquid filled into it during a normal assay, can be used with a displacer leaving 30—150 microlitre liquid spac , .g. 50—100 microlitre.